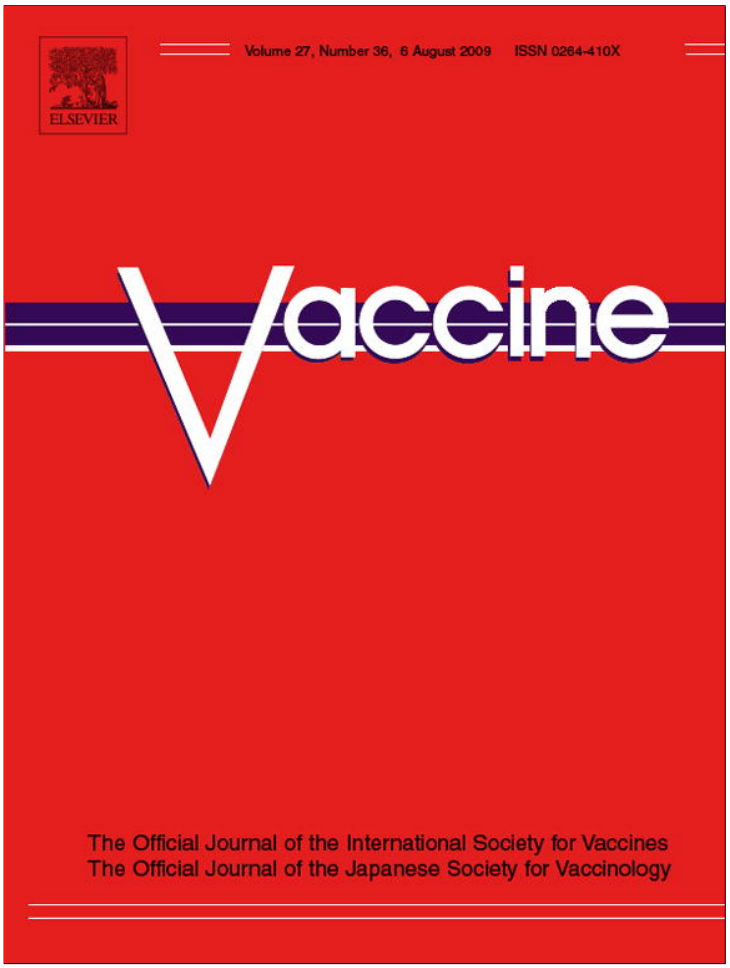

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
11 MAR 2009	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Immune interference after sequential alphavirus vaccine vaccination. Vaccine 27:4679-4882	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
Pittman PR Liu CT Cannon TL Mangiafico JA Gibbs PH	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT NUMBER
United States Medical Institute of Infectious Diseases, Fort Detrick, MD	**TR-08-086**
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES

The original document contains color images.

14. ABSTRACT

We compared the effect of order of administration of investigational alphavirus vaccines on neutralizing antibody response. Volunteers who received the inactivated eastern and western equine encephalitis (EEE and WEE) vaccines before live attenuated Venezuelan (VEE) vaccine had significantly lower rates of antibody response than those receiving VEE vaccine before EEE and WEE vaccines (66.7% vs. 80.6%; p=0.026). The odds of having a VEE antibody non-response among those initially receiving EEE and WEE vaccines, adjusted for gender, were significant (odds ratio [OR]=2.20; 95% CI=1.2-4.1 [p=0.0145]) as were the odds of non-response among females adjusted for group (OR=1.81; 95% CI=1.2-2.7 [p=0.0037]). Antibody interference and gender effect have major implications for vaccine strategy among those receiving multiple alphavirus vaccines and those developing next generation vaccines for these threats.

15. SUBJECT TERMS

Venezuelan equine encephalitis virus, VEE, vaccines, alphavirus, antibody responses, human studies

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**SAR**	**5**	

Vaccine 27 (2009) 4879–4882

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Short communication

Immune interference after sequential alphavirus vaccine vaccinations☆

Phillip R. Pittman[a,*], Ching-Tong Liu[a,1], Timothy L. Cannon[b], Joseph A. Mangiafico[a], Paul H. Gibbs[a]

[a] U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD 21702-5011, United States
[b] Information Support Division, Directorate of Information Management, Fort Detrick, MD 21702-5011, United States

ARTICLE INFO

Article history:
Received 21 November 2008
Received in revised form 23 February 2009
Accepted 24 February 2009
Available online 11 March 2009

Keywords:
Alphavirus
Eastern equine encephalitis
Western equine encephalitis
Venezuelan equine encephalitis

ABSTRACT

We compared the effect of order of administration of investigational alphavirus vaccines on neutralizing antibody response. Volunteers who received the inactivated eastern and western equine encephalitis (EEE and WEE) vaccines before live attenuated Venezuelan (VEE) vaccine had significantly lower rates of antibody response than those receiving VEE vaccine before EEE and WEE vaccines (66.7% vs. 80.6%; $p = 0.026$). The odds of having a VEE antibody non-response among those initially receiving EEE and WEE vaccines, adjusted for gender, were significant (odds ratio [OR] = 2.20; 95% CI = 1.2–4.1 [$p = 0.0145$]) as were the odds of non-response among females adjusted for group (OR = 1.81; 95% CI = 1.2–2.7 [$p = 0.0037$]). Antibody interference and gender effect have major implications for vaccine strategy among those receiving multiple alphavirus vaccines and those developing next generation vaccines for these threats.

1. Introduction

Venezuelan equine encephalitis (VEE), eastern equine encephalitis (EEE), and western equine encephalitis (WEE) are important causes of morbidity and mortality among humans and equids in the Western hemisphere [1]. All are caused by RNA viruses of the *Alphavirus* genus within the family Togaviridae [2,3]. Although the three diseases are acquired in nature after the bite of infected mosquitoes, the efficiency with which they can be transmitted via the aerosol route also makes them attractive candidates for use as biological weapons by adversary governments and/or terrorists [4–9].

For veterinary use, there are live, attenuated and inactivated VEE vaccines as well as inactivated vaccines for EEE and WEE [10]. However, there are no licensed EEE, WEE, or VEE vaccines marketed for use in humans. For more than 25 years, investigational vaccines against these agents have been administered at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) to laboratory workers and others at occupational risk for acquiring these infections. In 1973, Calisher et al. [11] demonstrated the phenomenon of vaccine interference in Texas horses receiving alphavirus vaccinations. McClain et al. [12] discovered immunologic suppression when live attenuated VEE and chikungunya (CHIK) virus vaccines were administered sequentially. In this report, we show that suppression of neutralizing antibody response to the live attenuated VEE vaccine can occur among volunteers who were previously vaccinated with eastern equine encephalitis and western equine encephalitis (EW) vaccines.

2. Methods

2.1. Volunteers and experimental design

Sources and preparation procedures of VEE, WEE, and EEE vaccines were described previously [13–16]. The VEE vaccine TC-83, attenuated, NDBR-12, Lot 4, was prepared at the National Drug Company in 1971. The virus was propagated in primary fetal guinea pig heart tissue culture maintained under Eagle's basal medium (BME) containing 50 $\mu g\,mL^{-1}$ each of neomycin and streptomycin and supplemented with 0.5% human serum albumin, U.S.P. The lyophilized vaccine is the filtered supernatant fluid harvested from cultures *ca* 30 h after infection and diluted to *ca* 10^4 p.f.u. $dose^{-1}$. The vaccine is stored at −20 °C. The lyophilized vaccine is reconstituted with sterile water for injection, U.S.P. and is administered by inoculating 0.5 mL of the vaccine subcutaneously.

The WEE vaccine, TSI-GSD-210 (lot 1–81), is a lyophilized product originating from the supernatant harvested from primary chicken fibroblast cell cultures. The vaccine was prepared from specific pathogen-free eggs infected with the attenuated CM4884 strain of WEE virus. The supernatant was harvested and filtered, and the virus was inactivated with formalin. The residual formalin was neutralized by sodium bisulfite and the supernatant was

☆ The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the Department of the Army or the Department of Defense.

* Corresponding author. Tel.: +1 301 619 2997; fax: +1 301 619 2312.
E-mail address: phillip.pittman@amedd.army.mil (P.R. Pittman).

[1] Deceased.

0264-410X/$ – see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.vaccine.2009.02.090

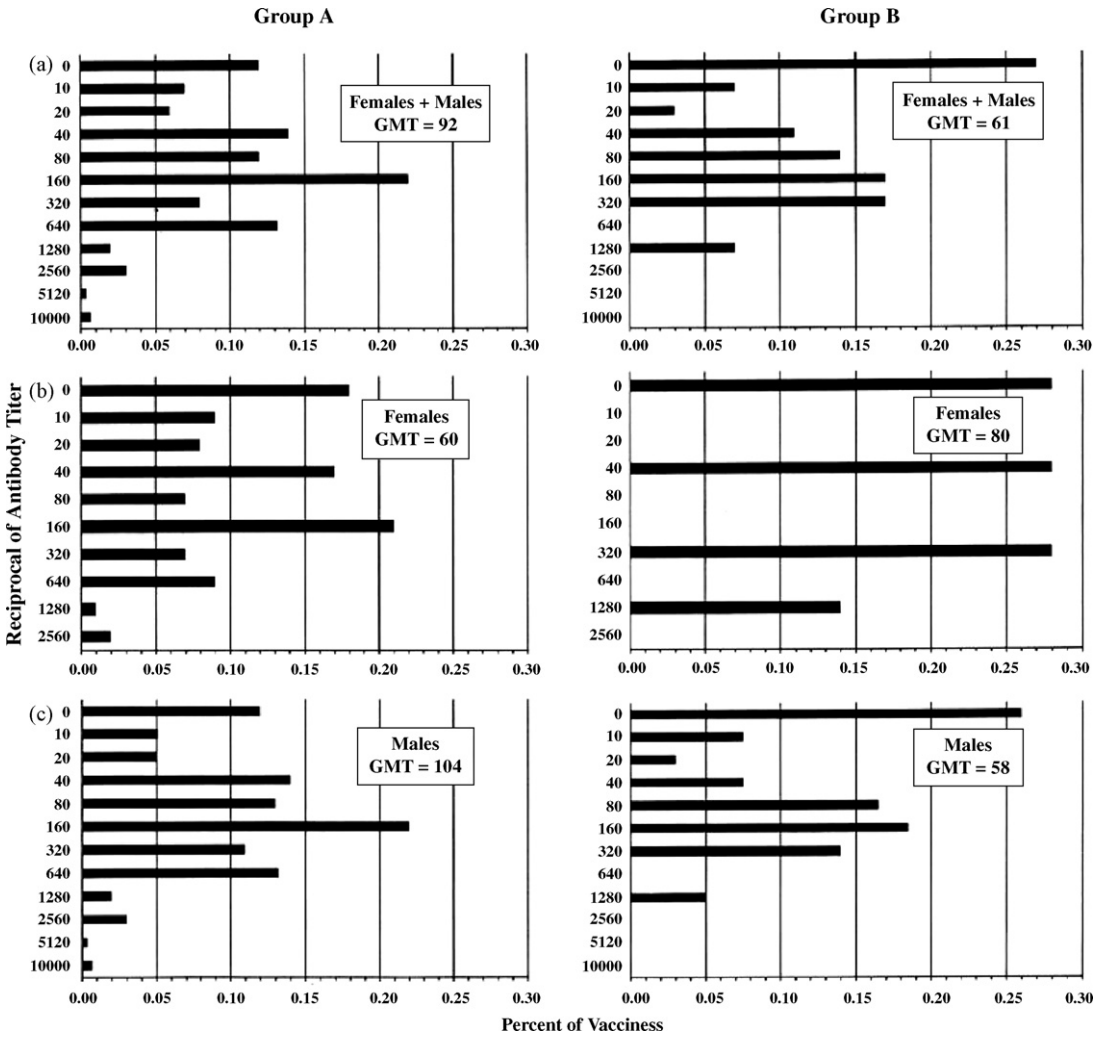

Fig. 1. Distribution of VEE neutralizing antibody titers and percent of vaccinees in groups A and B in which primary dose(s) of three alphavirus (EEE, WEE, and VEE) vaccines were administered in different sequences. Group A: VEE vaccine was administered and followed by titering for VEE. Afterward one or more doses of EEE or WEE may have been administered. Group B: EEE and WEE vaccines were administered first, followed by VEE vaccination, and titered for VEE. The GMTs (geometric mean titers) represent the total population (responders and non-responders) for the group. (a) Distribution of VEE neutralizing antibody titers for both genders in groups A and B. (b) Distribution of VEE neutralizing antibody titers for females in groups A and B. (c) Distribution of VEE neutralizing antibody titers for males in groups A and B.

lyophilized. The final product was manufactured at the Salk Institute, Government Services Division, Swiftwater, PA. The lyophilized vaccine was stored at −20 °C, reconstituted with 5 mL of sterile water for subcutaneous injection and used within 2 h of reconstitution. The primary vaccination series consists of 3 subcutaneous injections (0.5 mL) on days 0, 7, and 28.

The EEE vaccine, TSI-GSD-104 (lot 2-1-89), is a lyophilized product originating from the supernatant harvested from primary chicken embryo cell cultures. The vaccine was prepared from specific pathogen-free eggs infected with the attenuated PE-6 strain of EEE virus. The supernatant was harvested and filtered, and the virus was inactivated with formalin. The residual formalin was neutralized by sodium bisulfite and the supernatant was lyophilized. The final product was manufactured at the Salk Institute, Government Services Division, Swiftwater, PA. The lyophilized vaccine was stored at −20 °C, reconstituted with 5 mL of sterile water for subcutaneous injection and used within 2 h of reconstitution. The primary series consists of 3 subcutaneous injections (0.5 mL) on days 0, 7, and 28.

The study population consisted of 766 human volunteers in the Special Immunizations Program (SIP) at USAMRIID who received a single primary vaccination of an investigational live attenuated VEE vaccine (TC-83) [17] and priming doses of both inactivated investigational EEE and WEE vaccines in different sequences between 1 January 1976 and 30 April 1997. Written informed consent was obtained from each volunteer before receipt of any investigational vaccine.

For each vaccinee, dates of VEE, EEE, and WEE vaccinations were recorded in the SIP database. The chronological relationships between the EEE and WEE vaccination dates, and the VEE primary vaccination date, were used as criteria to allocate individuals into two groups. In group A, VEE vaccine was administered first, followed by EEE and WEE vaccines (alphavirus-naïves). In group B, EW vaccines were administered first, followed by VEE vaccination.

2.2. Serology

The neutralizing antibody titers to VEE vaccine were determined for both groups between 14 and 98 days after receipt of VEE vaccine, but before any subsequent EEE and WEE vaccines in group A. Antibodies were measured against Trinidad strain VEE virus, using a constant-virus, serum dilution technique as previously described [17]. A plaque reduction neutralization titer 80% ($PRNT_{80}$) ≥ 1:20 was considered positive for seroconversion.

Table 1
Overall VEE neutralizing antibody response rates of 766 volunteers who were administered VEE vaccine only (Group A) or VEE vaccine after EEE and WEE vaccines (Group B).

Group	Total vaccines (*N*)	Non-responders		Responders		GMT of responders (95% CL)[a]
		(*N*)	(%)	(*N*)	(%)	
A[b]	718	139	(19.4)	579	(80.6)	157 (141–174)
B[c]	48	16	(33.3)	32	(66.7)	150 (104–215)
p-Value		(*p* = 0.026)				(*p* = 0.845)

[a] CL = Confidence limits.
[b] Group A: VEE vaccine administered and titered. VEE vaccination may have been followed by one or more doses of EEE and/or WEE vaccines.
[c] Group B: EEE and WEE vaccinations first and followed by VEE vaccine then titered for VEE.

Table 2
VEE neutralizing antibody response rates by group, stratified by gender (*N* = 766).

	Gender	Total vaccinees (*n*) (%)	Non-response (*n*) (%)	Response (*n*) (%)	GMT of responders (95% CL)[a]
Group A	F	157 (22)	44 (28)	113 (72)	119 (95–150)
	M	561 (78)	95 (17)	466 (83)	168 (149–188)
Total		718 (100)	(*p* = 0.003)		(*p* = 0.01)
Group B	F	7 (15)	2 (29)	5 (71)	184 (49–686)
	M	41 (85)	14 (34)	27 (66)	144 (100–209)
Total		48 (100)			

[a] CL = Confidence limits.

2.3. Statistical analysis

Antibody response rates were compared using the Fisher's exact test, 2-tailed [18]. Geometric mean titers (GMTs) were compared by the Student's *t*-test and variance analysis (ANOVA). A standard error of the mean in terms of log values for GMT was calculated [19]. The null hypothesis was tested at the 95% confidence level. Multiple logistic regression (SAS) was used to build the response model to test the final model and give statistical estimates of the odds of being a non-responder (with 95% limits), adjusting for each other variable in the model.

3. Results

3.1. VEE neutralizing antibody response

The distribution of VEE neutralizing antibody titers expressed as the proportion of vaccinees in groups A and B is illustrated in Fig. 1a. When VEE vaccine was administered first, the antibody response rate was 80.6% (Table 1). In contrast, the antibody response rate was only 66.7% when subjects received EW vaccines before VEE ($p = 0.026$). There were no group differences in GMT against VEE among responders, regardless of group ($p = 0.845$).

3.2. Neutralizing antibody response rates: effects of age, gender, race, and vaccination sequence

Because this was an observational study and not a randomized trial, it was necessary to examine demographic factors as possible confounders with group membership. Fig. 1b and c show the distribution of VEE neutralizing antibody titers by group and gender. VEE neutralizing antibody response rates and GMTs are stratified by group and by gender in Table 2. For group A, the antibody response rate for males was 83%; females 72% ($p = 0.003$). Responder GMTs in group A also were lower in females than males ($p = 0.01$). Sample size for females ($N = 7$) was insufficient in group B for statistical gender comparisons. These results from group A imply that analysis of response to VEE TC-83 must account for gender before comparing groups.

By logistic regression analysis, gender and prior vaccination with EW were found to be jointly significant predictors of antibody non-response ($p = 0.0037$ and 0.0145, respectively) (Table 3). There was no statistical evidence of differences in odds of non-response due to age ($p = 0.75$), race ($p = 0.97$), or interaction between gender and vaccination sequence ($p = 0.27$). In a model adjusting for vaccination sequence, the odds ratio of non-response to VEE by gender (female vs. male) was 1.81 (95% CI = 1.2–2.7; $p = 0.0037$). When adjusting for gender, receipt of EEE and WEE before VEE yielded an OR of 2.20 for non-response in comparison with the group receiving VEE initially (95% CI = 1.2–4.1; $p = 0.0145$).

4. Discussion

These data indicate that vaccinating humans against EEE and WEE with inactivated vaccines may interfere with subsequent neutralizing antibody response to the live attenuated VEE TC-83 vaccine. This interference was manifested by the diminished ability to elicit an adequate antibody response of at least 1:20.

The phenomenon of alphavirus vaccine-induced interference was first observed in horses as a result of previous inoculations of EEE and WEE vaccines [11]. Similar immune suppression occurred in humans when two live alphavirus vaccines were administered sequentially in a study by McClain et al. [12]. In that study, a live attenuated CHIK vaccine was administered after inoculation of live attenuated VEE vaccine, TC-83, in humans or vice versa. Significant interference with the ability to elicit a neutralizing antibody response to the second vaccine was observed regardless of which vaccine was administrated first. The present study appears to be the first report of alphavirus vaccine interference, involving VEE, EEE, and WEE vaccines in humans. The exact mechanisms responsible for this interference are unknown. When we analyzed individuals who received VEE before EEE or WEE inactivated vaccines, we did not observe interference (data not shown). Several animal stud-

Table 3
Adjusted odds ratio (OR) of non-response (NR) by gender and EW vaccine exposure history.

	Adjusted data odds ratio	
	p-Value	OR (95% CL)
Female vs. male	0.0037	1.81 (1.2, 2.7)
Prior EW vs. no EW WEE before VEE	0.0145	2.20 (1.2, 4.1)

ies have shown cross-protection among alphaviruses [11,20–24]. Indeed, some of these studies showed that cross-protection may occur with non-neutralizing antibodies [23,24]. In our case, existing antibodies against EEE and WEE interfered with the development of antibodies against the live, attenuated VEE vaccine in humans.

In a previous report [17], we showed that females had a lower antibody response rate than males to the live attenuated VEE vaccine. The finding was reproduced in this study. During this time, VEE TC-83 live attenuated vaccine was administered to women during menstruation. Whether hormonal levels or some other mechanism is responsible for this phenomenon is unknown.

These data are important to medical researchers developing alphavirus vaccines and strategies for vaccination with multiple antigens.

Acknowledgements

The authors thank Drs. Kelly McKee and Ellen Boudreau for their critiques of the manuscript. Special acknowledgement is given to Alberta Blake, RN, who administered the vaccines in the Special Immunizations Clinic, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, between 1987 and 1999.

References

[1] Griffin DE. Alphaviruses. In: Knife DM, Howley PM, editors. Fields virology. 5th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2007. p. 1023–67.
[2] Kuhn RJ. Togaviridae: the viruses and their replication. In: Knife DM, Howley PM, editors. Fields virology. 5th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2007. p. 1001–22.
[3] Weaver SC, Frolov IV. Togaviruses. In: Mahy B, ter Meulen V, editors. Topley & Wilson's microbiology & microbial infections; virology. 5th ed. London: Hodder Arnold; 2005. p. 1010–24.
[4] Johnson KM, Martin DH. Venezuelan equine encephalitis. Adv Vet Sci Comp Med 1974;18:79–116.
[5] Johnson KM, Shelekov AP, Peralta PH, Dammin GJ, Young NA. Recovery of Venezuelan equine encephalomyelitis virus in Panama: a fatal case in man. Am J Trop Med Hyg 1968;17:432–40.
[6] Kubes V, Rios FA. The causative agent of infectious equine encephalomyelitis in Venezuela. Science 1939;90:20–1.
[7] Casals J, Curnen EC, Thomas L. Venezuelan equine encephalomyelitis in man. J Exp Med 1943;77:521–30.
[8] Lennett EH, Kaporowski H. Human infections with Venezuelan equine encephalitis virus. A report of eight cases of infection acquired in the laboratory. J Am Med Assoc 1943;123:1088–95.
[9] Slepushkin AN. An epidemiological study of laboratory infections with Venezuelan equine encephalitis. Prob Virol 1959;4:54–8.
[10] Minke JM, Audonnet J-C, Fischer L. Equine viral vaccines: the past, present, and future. Vet Res 2004;35:425–43.
[11] Calisher CH, Sasso DR, Sather GE. Possible evidence for interference with Venezuelan equine encephalitis virus vaccination of equines by pre-existing antibody to eastern or western equine encephalitis virus, or both. Appl Microbiol 1973;26:485–8.
[12] McClain DJ, Pittman PR, Ramsburg HH, Nelson GO, Rossi CA, Mangiafico JA, et al. Immunologic interference from sequential administration of live attenuated alphavirus vaccines. J Infect Dis 1998;177:634–41.
[13] Berge TO, Banks IS, Tigertt WD. Attenuation of Venezuelan equine encephalomyelitis virus by in vitro cultivation in guinea-pig heart cells. Am J Hyg 1961;73:209–18.
[14] McKinney RW, Berge TO, Sawyer WD, Tigertt WD, Crozier D. Use of attenuated strain of Venezuelan equine encephalomyelitis virus for immunization of man. Am J Trop Med Hyg 1963;12:597–603.
[15] Maire 3d LF, McKinney RW, Cole Jr FE. An inactivated eastern equine encephalomyelitis vaccine propagated in chick-embryo cell culture. I. Production and testing. Am J Trop Med Hyg 1970;190:119–22.
[16] Bartelloni PJ, McKinney RW, Calia FM, Ramsburg HH, Cole Jr FE. Inactivated western equine encephalomyelitis vaccine propagated in chick embryo cell culture: clinical and serological evaluation in man. Am J Trop Med Hyg 1971;20:146–9.
[17] Pittman PR, Makuch RS, Mangiafico JA, Cannon TL, Gibbs PH, Peters CJ. Long-term duration of detectable neutralizing antibodies after administration of live attenuated VEE vaccine and following booster vaccination with inactivated VEE vaccine. Vaccine 1996;14:337–43.
[18] SAS Institute. SAS/STAT User's Guide: release 6. 03 edition. Cary, NC: SAS Institute Inc.; 1988. pp. 1007–69.
[19] Snedecor GW, Cochran WG. Statistical methods applied to experiments in agriculture and biology. 5th ed. Ames, IA: Iowa State University Press; 1956. pp. 320–1.
[20] Cole Jr FE, McKinney RW. Cross-protection in hamsters immunized with group A arbovirus vaccines. Infect Immun 1971;4:37–43.
[21] Hearn Jr HJ. Cross-protection between Venezuelan equine encephalomyelitis and eastern equine encephalomyelitis virus. J Immunol 1961;107:607–10.
[22] Hearn HJ, Rainey CT. Cross-protection in animals infected with group A arboviruses. J Immunol 1963;90:720–4.
[23] Schmaljohn AL, Johnson ED, Dalrymple JM, Cole GA. Non-neutralizing monoclonal antibodies can prevent lethal alphavirus encephalitis. Nature 1982;297:70–2.
[24] Aguilar PV, Robich RM, Turell MJ, O'Guinn ML, Klein TA, Huaman A, et al. Endemic eastern equine encephalitis in the Amazon region of Peru. Am J Trop Med Hyg 2007;76:293–8.